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FILE 'HOME' ENTERED AT 13:50:56 ON 10 DEC 2008

| COST IN U.S. DOLLARS | SINCE FILE ENTRY | TOTAL SESSION |
|----------------------|------------------|---------------|
| FULL ESTIMATED COST | 1.05 | 1.05 |

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS, ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 13:53:46 ON 10 DEC 2008
ALL COPYRIGHTS AND RESTRICTIONS APPLY. SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s polyhydroxyalkan? or polyhydroxybuty? or pha or phb or 3hb co
FILE 'MEDLINE'

| | |
|--|--|
| 736 POLYHYDROXYALKAN? | |
| 263 POLYHYDROXYBUTY? | |
| 15727 PHA | |
| 1199 PHB | |
| 265 3HB | |
| 1459507 CO | |
| 99 3HB CO | |
| (3HB(W)CO) | |
| L1 17052 POLYHYDROXYALKAN? OR POLYHYDROXYBUTY? OR PHA OR PHB OR 3HB CO | |

FILE 'SCISEARCH'

| | |
|--|--|
| 1693 POLYHYDROXYALKAN? | |
| 718 POLYHYDROXYBUTY? | |
| 9302 PHA | |
| 2735 PHB | |
| 558 3HB | |
| 430899 CO | |
| 240 3HB CO | |
| (3HB(W)CO) | |
| L2 12439 POLYHYDROXYALKAN? OR POLYHYDROXYBUTY? OR PHA OR PHB OR 3HB CO | |

FILE 'LIFESCI'

| | |
|---|--|
| 648 POLYHYDROXYALKAN? | |
| 375 POLYHYDROXYBUTY? | |
| 6141 PHA | |
| 956 PHB | |
| 183 "3HB" | |
| 91399 "CO" | |
| 74 3HB CO | |
| ("3HB(W)"CO") | |
| L3 7215 POLYHYDROXYALKAN? OR POLYHYDROXYBUTY? OR PHA OR PHB OR 3HB CO | |

FILE 'BIOTECHDS'

| | |
|---|--|
| 895 POLYHYDROXYALKAN? | |
| 176 POLYHYDROXYBUTY? | |
| 842 PHA | |
| 782 PHB | |
| 113 3HB | |
| 13976 CO | |
| 45 3HB CO | |
| (3HB(W)CO) | |
| L4 1778 POLYHYDROXYALKAN? OR POLYHYDROXYBUTY? OR PHA OR PHB OR 3HB CO | |

FILE 'BIOSIS'

| | |
|------------------------|--|
| 1139 POLYHYDROXYALKAN? | |
| 441 POLYHYDROXYBUTY? | |

16550 PHA
1730 PHB
334 3HB
235132 CO
137 3HB CO
(3HB (W) CO)
L5 18648 POLYHYDROXYALKAN? OR POLYHYDROXYBUTY? OR PHA OR PHB OR 3HB CO

FILE 'EMBASE'
953 POLYHYDROXYALKAN?
269 POLYHYDROXYBUTY?
15385 PHA
1298 PHB
283 "3HB"
953748 "CO"
116 3HB CO
("3HB" (W) "CO")
L6 16889 POLYHYDROXYALKAN? OR POLYHYDROXYBUTY? OR PHA OR PHB OR 3HB CO

FILE 'HCAPLUS'
2659 POLYHYDROXYALKAN?
3786 POLYHYDROXYBUTY?
12555 PHA
4448 PHB
688 3HB
944433 CO
292 3HB CO
(3HB (W) CO)
L7 19524 POLYHYDROXYALKAN? OR POLYHYDROXYBUTY? OR PHA OR PHB OR 3HB CO

FILE 'NTIS'
8 POLYHYDROXYALKAN?
9 POLYHYDROXYBUTY?
417 PHA
39 PHB
0 3HB
36794 CO
0 3HB CO
(3HB (W) CO)
L8 461 POLYHYDROXYALKAN? OR POLYHYDROXYBUTY? OR PHA OR PHB OR 3HB CO

FILE 'ESBIOBASE'
790 POLYHYDROXYALKAN?
251 POLYHYDROXYBUTY?
4083 PHA
974 PHB
253 3HB
141122 CO
107 3HB CO
(3HB (W) CO)
L9 5129 POLYHYDROXYALKAN? OR POLYHYDROXYBUTY? OR PHA OR PHB OR 3HB CO

FILE 'BIOTECHNO'
527 POLYHYDROXYALKAN?
150 POLYHYDROXYBUTY?
4427 PHA
697 PHB
165 3HB
52091 CO
79 3HB CO
(3HB (W) CO)
L10 5240 POLYHYDROXYALKAN? OR POLYHYDROXYBUTY? OR PHA OR PHB OR 3HB CO

FILE 'WPIDS'

 732 POLYHYDROXYALKAN?
 830 POLYHYDROXYBUTY?
 1125 PHA
 573 PHB
 73 3HB
 320145 CO
 26 3HB CO
 (3HB(W)CO)

L11 2746 POLYHYDROXYALKAN? OR POLYHYDROXYBUTY? OR PHA OR PHB OR 3HB CO

TOTAL FOR ALL FILES

L12 107121 POLYHYDROXYALKAN? OR POLYHYDROXYBUTY? OR PHA OR PHB OR 3HB CO

=> s l12 and (feed rate# or ferment?(3a)parameter#)

FILE 'MEDLINE'

 52700 FEED
 1451183 RATE#
 337 FEED RATE#
 (FEED(W)RATE#)
 43706 FERMENT?
 416122 PARAMETER#
 255 FERMENT?(3A)PARAMETER#

L13 8 L1 AND (FEED RATE# OR FERMENT?(3A)PARAMETER#)

FILE 'SCISEARCH'

 89918 FEED
 1642916 RATE#
 3184 FEED RATE#
 (FEED(W)RATE#)
 61740 FERMENT?
 1047889 PARAMETER#
 670 FERMENT?(3A)PARAMETER#

L14 14 L2 AND (FEED RATE# OR FERMENT?(3A)PARAMETER#)

FILE 'LIFESCI'

 19500 "FEED"
 313973 RATE#
 316 FEED RATE#
 ("FEED"(W)RATE#)
 30002 FERMENT?
 99009 PARAMETER#
 309 FERMENT?(3A)PARAMETER#

L15 11 L3 AND (FEED RATE# OR FERMENT?(3A)PARAMETER#)

FILE 'BIOTECHDS'

 8392 FEED
 44316 RATE#
 664 FEED RATE#
 (FEED(W)RATE#)
 63559 FERMENT?
 11799 PARAMETER#
 427 FERMENT?(3A)PARAMETER#

L16 21 L4 AND (FEED RATE# OR FERMENT?(3A)PARAMETER#)

FILE 'BIOSIS'

 131104 FEED
 1534665 RATE#
 827 FEED RATE#
 (FEED(W)RATE#)
 91933 FERMENT?

```

451844 PARAMETER#
763 FERMENT?(3A)PARAMETER#
L17      11 L5 AND (FEED RATE# OR FERMENT?(3A)PARAMETER#)

FILE 'EMBASE'
21808 "FEED"
1276299 RATE#
512 FEED RATE#
("FEED"(W)RATE#)
32104 FERMENT?
453664 PARAMETER#
243 FERMENT?(3A)PARAMETER#
L18      9 L6 AND (FEED RATE# OR FERMENT?(3A)PARAMETER#)

FILE 'HCAPLUS'
265109 FEED
2390928 RATE#
12026 FEED RATE#
(FEED(W)RATE#)
205608 FERMENT?
127629 FERMN
238609 FERMENT?
(FERMENT? OR FERMN)
1285261 PARAMETER#
1465 FERMENT?(3A)PARAMETER#
L19      19 L7 AND (FEED RATE# OR FERMENT?(3A)PARAMETER#)

FILE 'NTIS'
15542 FEED
186859 RATE#
688 FEED RATE#
(FEED(W)RATE#)
2572 FERMENT?
136306 PARAMETER#
18 FERMENT?(3A)PARAMETER#
L20      0 L8 AND (FEED RATE# OR FERMENT?(3A)PARAMETER#)

FILE 'ESBIOBASE'
22097 FEED
467058 RATE#
383 FEED RATE#
(FEED(W)RATE#)
24039 FERMENT?
169550 PARAMETER#
324 FERMENT?(3A)PARAMETER#
L21      11 L9 AND (FEED RATE# OR FERMENT?(3A)PARAMETER#)

FILE 'BIOTECHNO'
5381 FEED
166001 RATE#
277 FEED RATE#
(FEED(W)RATE#)
23461 FERMENT?
60195 PARAMETER#
212 FERMENT?(3A)PARAMETER#
L22      6 L10 AND (FEED RATE# OR FERMENT?(3A)PARAMETER#)

FILE 'WPIDS'
525657 FEED
653015 RATE#
11922 FEED RATE#
(FEED(W)RATE#)

```

62798 FERMENT?
44 FERMN
62810 FERMENT?
(FERMENT? OR FERMN)
242647 PARAMETER#
111 FERMENT?(3A)PARAMETER#
L23 12 L11 AND (FEED RATE# OR FERMENT?(3A)PARAMETER#)

TOTAL FOR ALL FILES
L24 122 L12 AND (FEED RATE# OR FERMENT?(3A) PARAMETER#)

=> s l24 not 2004-2008/pY
FILE 'MEDLINE'
3257923 2004-2008/PY
(20040000-20089999/PY)
L25 4 L13 NOT 2004-2008/PY

FILE 'SCISEARCH'
6074569 2004-2008/PY
(20040000-20089999/PY)
L26 6 L14 NOT 2004-2008/PY

FILE 'LIFESCI'
776856 2004-2008/PY
L27 6 L15 NOT 2004-2008/PY

FILE 'BIOTECHDS'
119822 2004-2008/PY
L28 10 L16 NOT 2004-2008/PY

FILE 'BIOSIS'
2845241 2004-2008/PY
L29 6 L17 NOT 2004-2008/PY

FILE 'EMBASE'
2816842 2004-2008/PY
L30 6 L18 NOT 2004-2008/PY

FILE 'HCAPLUS'
6597712 2004-2008/PY
L31 10 L19 NOT 2004-2008/PY

FILE 'NTIS'
81634 2004-2008/PY
L32 0 L20 NOT 2004-2008/PY

FILE 'ESBIOBASE'
1609791 2004-2008/PY
L33 6 L21 NOT 2004-2008/PY

FILE 'BIOTECHNO'
586 2004-2008/PY
L34 6 L22 NOT 2004-2008/PY

FILE 'WPIDS'
5697821 2004-2008/PY
L35 6 L23 NOT 2004-2008/PY

TOTAL FOR ALL FILES
L36 66 L24 NOT 2004-2008/PY

=> dup rem 136

PROCESSING COMPLETED FOR L36

L37 22 DUP REM L36 (44 DUPLICATES REMOVED)

=> d tot

- L37 ANSWER 1 OF 22 BIOTECHDS COPYRIGHT 2008 THOMSON REUTERS on STN
TI Production of 2-hydroxyacid-containing polymer for forming medical device, involves expressing in organism exogenous genes encoding polyhydroxyalkanoate synthase and enzyme(s) for the production of 2-hydroxyacyl Coenzyme-A;
vector-mediated polyhydroxyalkanoate-synthase gene transfer and expression in host cell for recombinant protein production and polymer preparation
AU MARTIN D P; SKRALY F A
AN 2004-10980 BIOTECHDS
PI US 2003211131 13 Nov 2003
- L37 ANSWER 2 OF 22 BIOTECHDS COPYRIGHT 2008 THOMSON REUTERS on STN
TI Evaluation of spectrofluorometry as a tool for estimation in fed-batch fermentations;
Alcaligenes eutrophus fermentation for poly-beta-hydroxybutyrate production and process optimization
SO BIOTECHNOLOGY AND BIOENGINEERING; (2003) 83, 1, 104-111 ISSN: 0006-3592
AU HAGEDORN A; LEGGE RL; BUDMAN H
AN 2003-15154 BIOTECHDS
- L37 ANSWER 3 OF 22 BIOTECHDS COPYRIGHT 2008 THOMSON REUTERS on STN
TI Production of polyhydroxyalkanoate from starch and/or derivatives for making polymers and copolymers, by incubating an polyhydroxyalkanoate-producing microorganism in medium containing starch and/or derivatives;
polymer preparation by bacterium or yeast fermentation for drug delivery, orthopedic implant, tissue engineering and cardiovascular disorder therapy
AU LAPOINTE R; LAMBERT A; SAVARD L
AN 2002-17381 BIOTECHDS
PI US 2002031812 14 Mar 2002
- L37 ANSWER 4 OF 22 MEDLINE on STN DUPLICATE 1
TI Effect of *Vitreoscilla* hemoglobin biosynthesis in *Escherichia coli* on production of poly(beta-hydroxybutyrate) and fermentative parameters.
SO FEMS microbiology letters, (2002 Sep 10) Vol. 214, No. 2, pp. 223-7.
Journal code: 7705721. ISSN: 0378-1097.
AU Yu Huimin; Shi Yue; Zhang Yanping; Yang Shengli; Shen Zhongyao
AN 2002490888 MEDLINE
- L37 ANSWER 5 OF 22 HCPLUS COPYRIGHT 2008 ACS on STN
TI Quality control of polyhydroxyalkanoates in fed-batch culture based on a metabolic reaction model
SO Computer Applications in Biotechnology 2001: Modelling, Monitoring and Control of Biotechnical Processes, A Proceedings Volume from the IFAC International Conference, 8th, Quebec City, QC, Canada, June 24-27, 2001 (2002), Meeting Date 2001, 201-206. Editor(s): Dochain, Denis; Perrier, Michel. Publisher: Pergamon Press, Oxford, UK.
CODEN: 69DEI4; ISBN: 0-08-043681-1
AU Shimizu, Hiroshi; Chanprateep, Suchada; Hirunruttanakorn, Adisak; Kikuya, Kensuke; Shioya, Suteaki
AN 2002:784810 HCPLUS
DN 138:3707

- L37 ANSWER 6 OF 22 MEDLINE on STN DUPLICATE 2
TI Multivariable control of alcohol concentrations in the production of polyhydroxyalkanoates (PHAs) by *Paracoccus denitrificans*.
SO Biotechnology and bioengineering, (2001 Jul 20) Vol. 74, No. 2, pp. 116-24.
Journal code: 7502021. ISSN: 0006-3592.
AU Chanprateep S; Abe N; Shimizu H; Yamane T; Shioya S
AN 2001276729 MEDLINE
- L37 ANSWER 7 OF 22 WPIDS COPYRIGHT 2008 THOMSON REUTERS on STN
TI Commercial production of 4-hydroxybenzoic acid, useful in producing low cost liquid crystal polymers, comprises using mutant *Escherichia coli* cells, which overproduce chorismate, transformed with the pMCP2 plasmid
PI US 6030819 A 20000229 (200021)* EN 8[3]
WO 2000018943 A1 20000406 (200025) EN
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: JP SG
IN AMARATUNGA M; JOHNSON B F; LOBOS J H; WILLIAMS E D
- L37 ANSWER 8 OF 22 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 3
TI Effect of total nutrient feed on production of poly-3-hydroxybutyrate by *Methylobacterium* sp ZP24 grown on sugars
SO JOURNAL OF INDUSTRIAL MICROBIOLOGY & BIOTECHNOLOGY, (NOV 2000) Vol. 25, No. 5, pp. 276-279.
ISSN: 1367-5435.
AU Yellore V S; Ghatnekar M S; Pai J S; Desai A J (Reprint)
AN 2001:62630 SCISEARCH
- L37 ANSWER 9 OF 22 BIOTECHDS COPYRIGHT 2008 THOMSON REUTERS on STN
TI Continuous microbiological production of biodegradable polymer, e.g. polyhydroxybutyric acid;
poly-beta-hydroxybutyrate production
AU Babel W; Maskow T
AN 2000-01367 BIOTECHDS
PI DE 19820168 4 Nov 1999
- L37 ANSWER 10 OF 22 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 5
TI Estimation of residual biomass, PHB, and nutrient concentrations by supplied amount of ammonia solution in fermentation of *Alcaligenes latus*
SO JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY, (OCT 1999) Vol. 9, No. 5, pp. 554-561.
ISSN: 1017-7825.
AU Lee Y W (Reprint); Yamane T
AN 1999:836273 SCISEARCH
- L37 ANSWER 11 OF 22 MEDLINE on STN DUPLICATE 6
TI Closed-loop control of bacterial high-cell-density fed-batch cultures: production of mcl-PHAs by *Pseudomonas putida* KT2442 under single-substrate and cofeeding conditions.
SO Biotechnology and bioengineering, (1999 Nov 5) Vol. 65, No. 3, pp. 306-15.
Journal code: 7502021. ISSN: 0006-3592.
AU Kellerhals M B; Kessler B; Witholt B
AN 1999415709 MEDLINE
- L37 ANSWER 12 OF 22 HCPLUS COPYRIGHT 2008 ACS on STN
TI Studies on the production of poly-β-hydroxybutyric acid by fed-batch culture with DO-stat method employing *Alcaligenes eutrophus* mutant B510
SO Huanjing Xuebao (1999), 19(1), 6-10
CODEN: HKXUDL; ISSN: 0253-2468

AU Zhuang, Guoqiang; Li, Aiying; Wen, Xin; Qi, Qingsheng; Xu, Ping; Qu, Yinbo
AN 1999:104227 HCAPLUS
DN 130:236524

L37 ANSWER 13 OF 22 WPIDS COPYRIGHT 2008 THOMSON REUTERS on STN
TI Magnetic card, IC card processing equipment - has pulse switching circuit
that selects signals which are fed to external interruption type input
terminal element and card feed rate counter circuit of
CPU

PI JP 09062795 A 19970307 (199720)* JA 9[7]
JP 3178996 B2 20010625 (200138) JA 9
IN TOKITA M

L37 ANSWER 14 OF 22 MEDLINE on STN DUPLICATE 7

TI Experimental optimization of fed-batch culture for
poly-beta-hydroxybutyric acid production.

SO Biotechnology and bioengineering, (1997 Dec 20) Vol. 56, No. 6, pp.
697-705.

Journal code: 7502021. ISSN: 0006-3592.

AU Lee J H; Hong J; Lim H C
AN 2008464250 IN-PROCESS

L37 ANSWER 15 OF 22 BIOTECHDS COPYRIGHT 2008 THOMSON REUTERS on STN

TI Preparation of poly-beta-hydroxybutyric acid polymers;
systems control of *Methylobacterium extorquens* two-stage fermentation
on methanol with N-starvation

AU Groleau D; Bourque D; Pomerleau Y
AN 1995-12194 BIOTECHDS
PI US 5434062 18 Jul 1995

L37 ANSWER 16 OF 22 WPIDS COPYRIGHT 2008 THOMSON REUTERS on STN

TI Polyester compsn. comprising two poly:hydroxy-alkanoate cpds. - including
one in (semi)crystalline form, as nucleant, for paper, fabric, hygiene
articles, sustained drug or agrochemical release system, adhesive, etc.

PI WO 9428070 A1 19941208 (199503)* EN 18[0]
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL OA PT SE
W: AT AU BB BG BR BY CA CH CN CZ DE DK ES FI GB GE HU JP KG KP KR KZ
LK LU LV MD MG MN MW NL NO NZ PL PT RO RU SD SE SI SK TJ TT UA US
UZ VN

AU 9467275 A 19941220 (199512) EN
FI 9505645 A 19951123 (199607) FI
NO 9504748 A 19951123 (199607) NO
EP 700418 A1 19960313 (199615) EN [0]
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
JP 08510498 W 19961105 (199708) JA 22[0]
AU 683466 B 19971113 (199803) EN
US 5693389 A 19971202 (199803) EN 5[0]

IN LIGGAT J J

L37 ANSWER 17 OF 22 WPIDS COPYRIGHT 2008 THOMSON REUTERS on STN

TI New strain ATCC 55366 od *Methylobacterium extorquens* - producing high
yields of poly-beta-hydroxybutyrate polymer when grown on methanol

PI US 5302525 A 19940412 (199417)* EN 14[0]
CA 2083621 A 19940525 (199431)* EN
CA 2083621 C 20000620 (200043)* EN

IN BOURQUE D; GROLEAU D; POMERLEAU Y; POMERLEAU Y

L37 ANSWER 18 OF 22 BIOTECHDS COPYRIGHT 2008 THOMSON REUTERS on STN

TI Equipment and operation for fermentative PHB production using
gaseous substrate to guarantee safety from explosion;
poly-beta-hydroxybutyrate production by *Alcaligenes eutrophus* by gas
phase fermentation of hydrogen, oxygen and carbon dioxide

SO J.Chem.Eng.Jpn.; (1993) 26, 2, 225-27
 CODEN: JCEJAC
 AU Ishizaki A; Tanaka K; Takeshita T; Kanemaru T; Shimoji T; Kawano T
 AN 1993-07106 BIOTECHDS

L37 ANSWER 19 OF 22 BIOTECHDS COPYRIGHT 2008 THOMSON REUTERS on STN
 TI Physiologically motivated strategies for control of the fed-batch cultivation of recombinant Escherichia coli for phenylalanine production; effect of glucose feeding, tyrosine feeding, oxygen supply; fed-batch culture; systems control

SO J.Ferment.Bioeng.; (1991) 71, 5, 350-55
 CODEN: JFBIEX
 AU Konstantinov K B; Nishio N; Seki T; *Yoshida T
 AN 1991-10001 BIOTECHDS

L37 ANSWER 20 OF 22 LIFESCI COPYRIGHT 2008 CSA on STN
 TI Characterization of intracellular accumulation of poly- beta -hydroxybutyrate (PHB) in individual cells of Alcaligenes eutrophus H16 by flow cytometry.

SO BIOTECHNOL. BIOENG., (1984) vol. 26, no. 8, pp. 982-987.
 AU Srienc, F.; Arnold, B.; Bailey, J.E.
 AN 84:26678 LIFESCI

L37 ANSWER 21 OF 22 HCPLUS COPYRIGHT 2008 ACS on STN
 TI Extraction of poly(β -hydroxybutyric acid)
 SO Brit. UK Pat. Appl., 4 pp.
 CODEN: BAXXDU
 IN Walker, John
 AN 1982:545715 HCPLUS
 DN 97:145715
 OREF 97:24295a, 24298a

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---------------|-------|----------|-----------------|----------|
| ----- | ----- | ----- | ----- | ----- |
| PI GB 2089823 | A | 19820630 | GB 1981-35734 | 19811126 |
| GB 2089823 | B | 19840627 | | |

L37 ANSWER 22 OF 22 HCPLUS COPYRIGHT 2008 ACS on STN
 TI Extraction of poly(3-hydroxybutyric acid) from microbial cells
 SO Eur. Pat. Appl., 20 pp.
 CODEN: EPXXDW
 IN Holmes, Paul Arthur; Wright, Leonard Frederick; Alderson, Barry; Senior, Peter James
 AN 1980:619324 HCPLUS
 DN 93:219324
 OREF 93:35023a, 35026a

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|-----------------------------------|-------|----------|-----------------|----------|
| ----- | ----- | ----- | ----- | ----- |
| PI EP 15123 | A1 | 19800903 | EP 1980-300431 | 19800214 |
| EP 15123 | B1 | 19821222 | | |
| R: BE, CH, DE, FR, GB, IT, LU, NL | | | | |
| ZA 8000803 | A | 19810624 | ZA 1980-803 | 19800212 |
| EP 36699 | A1 | 19810930 | EP 1981-200352 | 19800214 |
| EP 36699 | B1 | 19830202 | | |
| EP 36699 | B2 | 19870902 | | |
| R: BE, CH, DE, FR, GB, IT, LU, NL | | | | |
| AU 8055606 | A | 19800828 | AU 1980-55606 | 19800215 |
| AU 529981 | B2 | 19830630 | | |
| DK 8000733 | A | 19800822 | DK 1980-733 | 19800220 |
| JP 55118394 | A | 19800911 | JP 1980-21041 | 19800221 |
| RO 79661 | A1 | 19820817 | RO 1980-100264 | 19800221 |
| US 4324907 | A | 19820413 | US 1980-125483 | 19800222 |

=> d ab 2-6, 8-12, 14, 15, 18-20

L37 ANSWER 2 OF 22 BIOTECHDS COPYRIGHT 2008 THOMSON REUTERS on STN
AB AUTHOR ABSTRACT - Native culture fluorescence was investigated as an additional source of information for predicting biomass and glucose concentrations in a fed-batch fermentation of *Alcaligenes eutrophus*. Partial least squares (PLS) regression and a feed forward neural network (FFNN) coupled with principle component analysis (PCA) were each used to model the kinetics of the fermentation. Data from three fermentations was combined to form a training set for model calibration and data from a fourth fermentation was used as the testing set. The fluorescent soft-sensors were compared with a previously developed feed forward neural network soft-sensor model which used oxygen uptake rate (OUR), carbon dioxide evolution rate (CER), aeration rate, feed rate, and fermentor volume to estimate biomass and glucose concentrations. The best model performance for predicting both biomass and glucose concentrations was achieved using the native fluorescence-based models. Real data predictions of the biomass concentration in the testing set were obtained using both the PLS and FFNN PCA modeling utilizing fluorescence measurements plus the rate of change of the fluorescence measurements. Accurate predictions of the glucose concentration in the testing set were obtained using the FFNN PCA modeling technique utilizing the rate of change of the fluorescence measurements. Substrate exhaustion was indicated qualitatively by a first-order PLS model utilizing the rate of change of fluorescence measurements. These results indicate that native culture fluorescence shows promise for providing additional valuable information to enhance predictive modeling which cannot be extracted from other easily acquired measurements. (C) 2003 Wiley Periodicals, Inc. (8 pages)

L37 ANSWER 3 OF 22 BIOTECHDS COPYRIGHT 2008 THOMSON REUTERS on STN
AB DERWENT ABSTRACT:

NOVELTY - Production of polyhydroxyalkanoate (PHA) from starch and/or derivatives by incubating a strain of PHA-producing microorganism for a sufficient period of time and conditions (i.e. fermentation conditions) to produce the PHA in a culture medium containing starch and/or their derivatives.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a PHA produced by incubation of a strain of PHA-producing microorganism in a culture medium comprising starch and/or their derivative.

BIOTECHNOLOGY - Preferred Method: In the process, the starch is isolated from a starch-containing biomass which is preferably from plant, waste water, wash water, potato, and a by-products or its derivative, where the biomass is processed to render the starch sufficiently available to be chemically, biochemically, biologically or enzymatically treated preferably the starch is hydrolyzed starch. The process to render the starch sufficiently available from the processed biomass is by homogenizing, grinding, crushing, shredding, cutting up, carving, breaking, solubilizing, lyophilizing, digesting, fermenting, incubating, dessicating, microbiologically treating, thermally treating, chemically treating, biochemically treating, and biologically treating the starch or their combination. The microorganism is from bacteria, mold, yeast, *Azotobacter*, *Pseudomonas*, *Nocardia*, *Coliform*, *Alcaligenes*, *Bacillus*, *Lactobacillus*, *Burkholderia*, *Rhodococcus* and *Methylobacterium* or their genetically modified forms. The method further comprises isolating PA from the microorganism and/or the medium.

USE - The PHA is useful for producing a polymer or copolymer such as polyesters, copolyesters, polyester-carbonase, and polyester-urethanes. PHA is biocompatible, hence is utilized in drug delivery, orthopedic implant, tissue engineering and cardiovascular

uses.

EXAMPLE - An inoculum of a Azotobacter salinestrus (strain ATCC 49674) was grown aerobically in a medium that contained the following mineral salts: 0.6-3.0 mM magnesium sulfate, 10-200 μM ferrous sulfate, 1.0-6.0 mM potassium phosphate monobasic or 2-5 mM potassium phosphate dibasic, 0.7-32 μM sodium molybdate, 10-25 mM sodium chloride, and 0.4-1 mM calcium sulfate and calcium chloride. The resulting inoculum was then added to a bioreactor (CHEMAP) containing a fermentation medium. The fermentation was carried out at 30°C in a fed-batch mode at pH 7 using concentrated solution of sodium hydroxide or sulfuric acid. The aeration rate and the agitation speed were adjusted manually during course of fermentation to maintain the level of oxygen above 5% and below 30% saturation. The maximum agitation speed reached was 610 rpm. Foam formation was controlled with addition of MAZU. Glucose was fed throughout growth phase from 20-80% w/v stock solution as obtained by starch hydrolysis, at a rate of approximately 5-10 ml/l/h. Spent nutrients were provided throughout growth phase by feeding a 4-20 times concentrated fermentation medium. Feed rate was approximately 5-10 ml/h. The fermentation was stopped after 30 hours. Polyhydroxyalkanoate (PHA) was recovered using modified methods of Berger et. al., (1989) Biotechnology Techniques, 3:227-232. Cells were centrifuged and then washed twice in distilled water. Fifty ml of methanol were added to an equivalent of 5 g (dry weight) of cells and vigorously mixed. The mixture was incubated at 40°C and the cells were harvested by centrifugation. The supernatant was discarded and chloroform was added to the pellet. The mixture was gently agitated and incubated at 40°C. One hundred ml of distilled water was added to the chloroform mixture, carefully agitated and centrifuged. The lower phase was recuperated and the soluble polymer precipitated with the addition of cold ethanol 95% under continuous agitation. The precipitated PHA obtained was recovered by filtration and dried at room temperature avoiding light exposure. At the end of the fermentation, the cell biomass concentration was 30-40 g/l (dry weight), containing approximately 15-20 g/l of poly(hydroxybutyrate-co-hydroxyvalerate) PHB/HV (92% HB and 8% HV) with a molecular weight of 1 million and polydispersity index of 1.2. (7 pages)

- L37 ANSWER 4 OF 22 MEDLINE on STN DUPLICATE 1
AB In order to attain high cell density and low cost production of poly(beta-hydroxybutyrate) (PHB), the *Vitreoscilla* globin gene (*vgb*) was introduced into a novel recombinant strain, *Escherichia coli* VG1 (pTU14). Experiments showed that the expression of *vgb* was under the regulation of dissolved oxygen (DO) in broth and the introduction of *vgb* in VG1 (pTU14) induced the parent promotion effect on cell growth and PHB accumulation, especially under low DO conditions. Further experiments indicated that the introduction of *vgb* in VG1 (pTU14) not only decreased the critical oxygen concentration, but also affected the volumetric oxygen transfer coefficient of the recombinant strain.
- L37 ANSWER 5 OF 22 HCPLUS COPYRIGHT 2008 ACS on STN
AB In order to control the mole fraction of monomer unit of poly(3-hydroxybutyrate-co-3-hydroxyvalerate), P(3HB-co-3HV), a biodegradable polymer, a novel multivariable control strategy is developed in a fed-batch culture of *Paracoccus denitrificans* ATCC 17741. This controller consists of two parts: one is for model predictive ethanol concentration control and the other one is for mole fraction control of monomer units. When the mole fraction of 3HV units has a target value, the feed rate of n-pentanol becomes a function of the feed rate of ethanol and the set value of 3HV, based on a metabolic reaction (MR) model. The mole fraction of 3HV units successfully reaches to the target value using this strategy. Chemical distribution is also analyzed based on the first order Marcovian

statistics.

- L37 ANSWER 6 OF 22 MEDLINE on STN DUPLICATE 2
- AB A novel multivariable control strategy is developed for alcohol (ethanol and n-pentanol) concentrations in the production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate), P(HB-co-HV), a biodegradable polymer by *Paracoccus denitrificans* ATCC 1774. This controller, which is developed to control the mole fraction of P(HB-co-HV), consists of two parts: one is for ethanol concentration control and the other is for mole fraction control, based on the concept of metabolic flux distribution control. A simple metabolic reaction (MR) model is constructed for flux distribution analysis. The relationship between mole ratio of specific consumption rate of the two alcohols (ethanol and n-pentanol) and the mole fraction of 3HV units in the polymer is linear. This result suggests that the split ratio at a branched point of 3-ketovaleryl-CoA in the P(HB-co-HV) synthetic pathway is constant for several fermentation conditions. When the mole fraction of 3HV units has a target value, the feed rate of n-pentanol becomes a function of the feed rate of ethanol and the set value of 3HV, based on the MR model. The mole fraction of 3HV units successfully reached the target value using this strategy. The mole fraction control strategy is combined with an optimal production strategy based on the optimal profile of the specific growth rate. The combined strategy is realized using multivariable controllers and P(3HB-co-3HV) production is maximized with a given value of mole fraction of 3HV units at the final step of fermentation.
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- L37 ANSWER 8 OF 22 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 3
- AB *Methylobacterium* sp. ZP24 is able to produce poly-3-hydroxybutyrate (PHB) from sucrose and lactose. As the production of PHB is growth-associated, a strategy of intermittent feeding of sugars and other nutrients was assessed for obtaining high yields of the polymer. Higher PHB synthesis was obtained at increased sugar feed rates. Cellular PHB contents of 63% and 71%, with productivities of up to 0.354 and 0.645 g PHB/I h were obtained from sucrose and lactose, respectively. A short-duration semicontinuous production level of up to 2.4 g PHB/I h was achieved in the lactose fermentation.

- L37 ANSWER 9 OF 22 BIOTECHDS COPYRIGHT 2008 THOMSON REUTERS on STN
- AB A new continuous culture method for producing poly-beta-hydroxybutyrate (PHB) involves growing a suitable microorganism (*Comamonas acidovorans*, *Comamonas testosteroni*, *Ralstonia eutropha* or *Variovorax paradoxus*) on a substrate at a feed rate giving maximum heat production. The PHB producer strains are grown chemostatically at maximum heat production on substrates which exhibit inhibition of growth with excess substrate. The maximum heat production corresponds to a maximum PHB content in the biomass and is controlled by controlling the rate of throughput of the substrate. The PHB formed is a biodegradable plastic polymer. The method allows continuous production of a useful product using ecotoxic by-products from the chemical industry such as phenols and benzoates as C-source for selected microorganisms. PHB formation is initiated and controlled so as to increase the substrate throughput rate while maintaining low volume interchange. In an example, *R. eutropha* JMP 134 was grown and the yield of PHB was 17% of the dry weight of the biomass. (3pp)

- L37 ANSWER 10 OF 22 SCISEARCH COPYRIGHT (c) 2008 The Thomson Corporation on STN DUPLICATE 5

AB A novel estimation method was investigated for determining the concentrations of residual biomass, poly-3-hydroxybutyrate (PHB), and main nutrients including carbon and nitrogen sources, phosphate, and mineral ions from the supplied amount of ammonia solution used for a pH-control solution and nitrogen source in a PHB fermentation. The estimation equations for a batch culture and a fed-batch culture were derived from the relationship between the growth rate of residual biomass and the feed rate of the pH-control solution, and then were applied to the batch culture and the fed-batch cultures of *Alcaligenes latus*. This method was successfully applied to estimate the concentrations of residual biomass, PHB, and nutrients.

L37 ANSWER 11 OF 22 MEDLINE on STN DUPLICATE 6
AB *Pseudomonas putida* KT2442 is able to accumulate medium-chain-length poly(3-hydroxyalkanoates) (mcl-PHAs) as intracellular inclusions on a variety of fatty acids and many other carbon sources. Some of these substrates, such as octanoic acid, alkenoic acids, and halogenated derivatives, are toxic when present in excess. Efficient production of mcl-PHAs on such toxic substrates therefore requires control of the carbon source concentration in the supernatant. In this study, we develop a closed-loop control system based on on-line gas chromatography to maintain continuously fed substrates at desired levels. We used the graphical programming environment LABVIEW to set up a flexible process control system that allows users to perform supervisory process control and permits remote access to the fermentation system over the Internet. Single-substrate supernatant concentration in a high-cell-density fed-batch fermentation process was controlled by a proportional (P) controller ($P = 50\%$) acting on the substrate pump feed rate. Na-octanoate concentrations oscillated around the setpoint of 10 mM and could be maintained between 0 and 25 mM at substrate uptake rates as high as 90 mmol L⁻¹ h⁻¹. Under ccofeeding conditions Na-10-undecenoate and Na-octanoate could be individually controlled at 2.5 mM and 9 mM, respectively, by applying a proportional integral (PI) controller for each substrate. The resulting copolymer contained 43.5 mol% unsaturated monomers and reflected the ratio of 10-undecenoate in the feed. It was suggested that both substrates were consumed at similar rates. These results show that this control system is suitable for avoiding substrate toxicity and supplying carbon substrates for growth and mcl-PHA accumulation.
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L37 ANSWER 12 OF 22 HCPLUS COPYRIGHT 2008 ACS on STN
AB Glucose can be utilized by *A. eutrophus* mutant B510 to accumulate poly- β -hydroxybutyric acid (PHB) in mineral medium. In order to eliminate the substrate inhibition and obtain an optimal substrate feed rate in a fed-batch culture with B510, a mathematic model reflecting the relationship between the specific growth rate and substrate concentration was developed and the growth yield was calculated. An optimal feed rate (13 g/L signal) for cell growth and an optimal substrate concentration [35 g/L of glucose and 1.6 g/L of (NH₄)₂SO₄] for PHB accumulation were determined. The final cell biomass and PHB production in the DO-stat fed-batch culture were 80 g/L and 62 g/L, resp.

L37 ANSWER 14 OF 22 MEDLINE on STN DUPLICATE 7
AB The optimal feed rate profiles of glucose and ammonium hydroxide were calculated using a proposed model, and implemented for the production of poly-beta-hydroxybutyric acid (PHB) by *Alcaligenes eutrophus*. By implementing these optimal feed rates with a high glucose feed concentration of 700 g/L and an ammonium hydroxide concentration of 7% (w/w), it was possible to achieve a high

final cell concentration of 141 g/L and a high PHB concentration of 105 g/L in 40 h of fed-batch operation. The PHB productivity was as high as 2.63 g/(L hr). (c) 1997 John Wiley & Sons, Inc. Biotechnol Bioeng 56: 697-705, 1997.

- L37 ANSWER 15 OF 22 BIOTECHDS COPYRIGHT 2008 THOMSON REUTERS on STN
AB Poly-beta-hydroxybutyrate (PHB) may be produced by aerobic culture of *Methylobacterium extorquens* ATCC 55366 in an aqueous culture medium with an assimilable C-source (e.g. methanol) and N-source, then in a 2nd stage under N-starvation when the cell dry weight is 20-90 g/l. Compressed air is used as the oxygen source, and the pH is controlled by addition of a base containing assimilable N (e.g. ammonium hydroxide) during the 1st stage, and an N-free base (e.g. potassium hydroxide) during N-starvation. Equations for control of the feed rate for addition of C-source in response to the dissolved oxygen level are presented. The mol.weight of the PHB product is 100,000-2,000,000. In an example, *M. extorquens* was grown under an imposed N-limitation during the PHB accumulation phase. After 93.5 hr, the culture contained 95.8 g/l dry weight cell biomass, containing 40.1% PHB of mol.weight 300,000. (15pp)
- L37 ANSWER 18 OF 22 BIOTECHDS COPYRIGHT 2008 THOMSON REUTERS on STN
AB *Alcaligenes eutrophus* ATCC 17697 was used for the production of poly-beta-hydroxybutyrate (PHB) from a mixture of hydrogen, oxygen and carbon dioxide using conditions that maintained the O₂ concentration at below the lower limit of explosion (6.9% O₂). The bench-scale fermentation system incorporated a 2 l jar fermentor. The gas mixture (H₂:CO₂ = 9:1) was stored in an iron-bell gas chamber (450 l) standing in saline solution, and was fed into the fermentor through a sterile filter by a circulating pump, and then returned to the chamber. Any change in pressure within the chamber due to gas consumption was compensated by rise of the saline water. Pure O₂ gas was directly fed into the medium independently from the mixed gas. An internal pressure sensor connected to a gas releasing system and a combustible gas detector interfaced with a shut-down device were also installed. A turbine impellor with 6 plane blades was used to obtain high kLa values. Fermentation was performed at 30 deg with agitation at 700 rpm and a gas feed rate of 2 l/min in a working volume of 1 ml. The PHB yield reached 61.5 g/l, at a maximum productivity of 2.84 g/l.hr. (12 ref)
- L37 ANSWER 19 OF 22 BIOTECHDS COPYRIGHT 2008 THOMSON REUTERS on STN
AB The effects of glucose feeding, tyrosine feeding and oxygen supply on the fed-batch cultivation of recombinant *Escherichia coli* AT2471 for the production of phenylalanine (PHA) were examined. The efficiency of the process was highly dependent on the distribution of the C flow between the main process products - biomass, PHA, acetic acid and CO₂. A set of control strategies was developed, designed to tolerate PHA biosynthesis at the expense of the remaining products. The dissolved oxygen concentration was controlled to prevent acetic acid secretion due to oxygen limitation. The total amount of tyrosine fed was used to provide an optimal balance between biomass production and that of PHA. Special algorithms for control of the glucose feed rate were applied to eliminate the possibility of acetic acid secretion due to overfeeding and to reduce excessive CO₂ evolution caused by severe glucose limitation. The application of these strategies resulted in greatly improved efficiency in the production of PHA. The final PHA concentration reached was 46 g/l, the yield above 17%, and the productivity 0.85 g/l.hr. (14 ref)

AB The analysis of a growing microbial population by flow cytometry offers several advantages over traditional methods for determining fermentation parameters. Poly- beta -hydroxybutyrate (PHB) accumulates in individual cells of Alcaligenes eutrophus in the form of refractile bodies which alter the light-scattering properties of individual cells. Flow cytometry has been applied to measure the distributions of single-cell light-scattering intensity in Alc. eutrophus populations during batch cultivation of the organism. These measurements clearly identify heterogeneities in the inoculum which influence the lag interval prior to beginning of exponential growth.

=> log y

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